Mapping of mutants resistant to p-fluorophenylalanine in diploid *Aspergillus nidulans*, lethal in haploids

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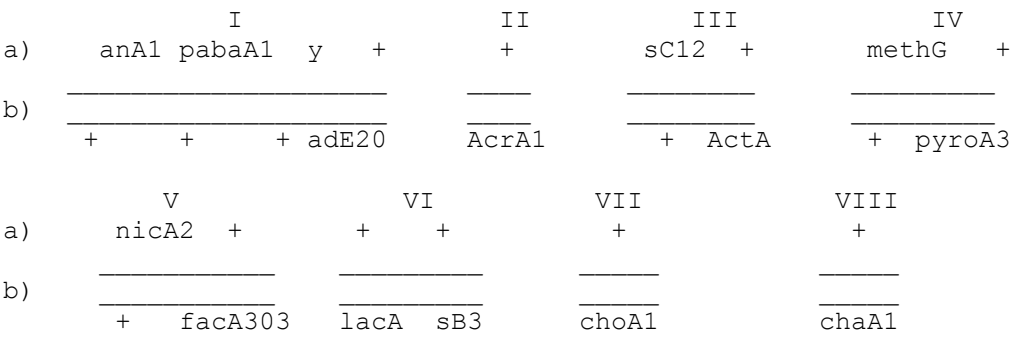
. In a previous paper (Babudri and Morpurgo 1990 Curr. Genet. 17:519-522) we described a new class of para-fluorophenylalanine (FPA) resistant mutants in *Aspergillus nidulans*. These mutants were obtained by plating UV irradiated diploid conidia on minimal medium (MM) supplemented with FPA (0.188 mg/ml). Some of them have characteristics different from those previously described, showing resistance to FPA in the diploid strains and being lethal in hemizygous or homozygous condition, either in presence or absence of FPA. This means that the genes involved affect fundamental processes of the fungus and at the same time, can mutate to give FPA resistance. The diploid strain we used was not marked on all eight chromosomes of *A. nidulans* and it was impossible to map all the lethal mutants; we have therefore synthesized a new diploid using our strain 35 (*anA1 pabaA1 yA2;sC12;methG1;nicA2*) and the tester strain FGSC 513 (*adE20;AcrA1;ActA1;pyroA3;facA303;lacA1 sB3;choA1;chaA1*) which allows precise chromosomal mapping. We irradiated the conidia with weak doses of UV rays and selected diploid resistant mutants on MM plus FPA. These were haploidized by seeding conidia on MM supplemented with sublethal doses of Benomyl. The microcolonies growing in the Benomyl supplemented medium were transferred on complete medium (CM) and all the segregant sectors were analyzed, testing the FPA resistance. Ten out of thirteen selected produced rare FPA resistant mutants, presumably haploid, all requiring pyridoxine and methionine (*methG1* and *pyroA3* map on chromosome IV). These mutants were not further analyzed because it is possible that they have an impaired capacity for uptake of amino acids as do some FPA resistant mutants isolated by Sinha (1967 Genet. Res. 10:261-272) and Tiwary et al. (1987 Curr. Microbiol. 15:305-311). The mutants 302-11, 302-12 and 302-15 failed to produce haploid FPA resistant segregants even if the colonies grown on the Benomyl supplemented medium were transferred to CM supplemented with FPA on which the resistant segregants could be selected. The analysis of the FPA sensitive sectors gave the following results: 302-11 and 302-15 map to chromosome VIII (Table 1). The situation of 302-12 is more complicated. The diploid FPA resistant strain does not utilize lactose and requires thiosulphate and is therefore a non-disjunctional diploid homozygous for chromosome VI. The analysis of the sectors mapped the gene on chromosome IV; all the sectors required methionine and this excludes the possibility that the mutant has impaired uptake of amino acids. It is not known if the requirement for thiosulphate is involved in FPA resistance but it is noteworthy that a mutant diploid described in the previous paper is thiosulphate requiring. Considering these results and those obtained in the previous work, three loci mapping to chromosomes I, IV and VIII which determine high level FPA resistance and are lethal in haploids have been identified. Research is in progress to determine 1) how many different loci may exhibit these characteristics and 2) the physiological basis of the phenomenon. This second point may be achieved by looking for temperature sensitive conditional lethals.

Table 1. Mapping of the FPAR lethals in *Aspergillus nidulans* by the diploid 35/513 ("a" and "b" respectively).



In haploids the segregation of the chromosomes should be random. The mapping of FPAR lethals is determined by the absence of a chromosome in a translocation free diploid strain. In the strain 302-11, chromosome VIIIb was missing in the 32 haploid segregants tested. The same chromosome was missing from the 37 haploid sectors tested in the strain 302-15. In the strain 302-12, all the haploid sectors lacked chromosome IVb. The FPAR mutant nø1 (see Babudri and Morpurgo, 1990) was obtained in a different diploid but the method used for mapping was the same; this mutant was mapped on chromosome I.